

CLAIMS

1. A method of detecting a nucleic acid,
comprising the steps of:

- 5 (1) preparing a single-stranded nucleic acid
having plural partial and sequential base sequences
to be detected (A-strand) and a single-stranded
nucleic acid having a base sequence complementary to
a base sequence of the A-strand (B-strand);
- 10 (2) preparing nucleic acids as primers each
having one of the plural base sequences to be
detected, immobilizing the respective primers
independently in separate regions on a substrate, and
preparing a primer array in which the respective base
sequences to be detected are distributed in the
15 primer-immobilized regions;
- (3) preparing a nucleic acid having a sequence
complementary to a partial and sequential base
sequence within the region between a 3'-end of the A-
strand and the base sequence to be detected which is
20 located nearest the 3'-end as a primer for elongating
the B-strand;
- (4) performing PCR reactions using the A-strand
and B-strand as templates, and using the primers
immobilized on the substrate, and the primer for
25 elongating the B-strand;
- (5) forming a hybridized product of a nucleic
acid corresponding to the A-strand which has been

elongated and amplified as a result of the PCR reactions and bound to the substrate and a nucleic acid corresponding to the B-strand which has been elongated and amplified and has not bound to the
5 substrate; and

(6) detecting the base sequence to be detected by detecting the hybridized product in the respective primer-immobilized regions in the array.

2. A method of detecting a nucleic acid,
10 comprising the steps of:

(1) preparing a single-stranded nucleic acid having plural partial and sequential base sequences to be detected (A-strand) and a single-stranded nucleic acid having a base sequence complementary to
15 a base sequence of the A-strand (B-strand);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and
20 preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing a nucleic acid having a partial and sequential base sequence within the region
25 between a 5'-end of the A-strand and the base sequence to be detected which is located nearest the 5'-end as a primer for elongating the A-strand and

preparing a nucleic acid having a sequence
complementary to a partial and sequential base
sequence within the region between a 3'-end of the A-
strand and the base sequence to be detected which is
5 located nearest the 3'-end as a primer for elongating
the B-strand;

(4) performing PCR reactions using the A-strand
and B-strand as templates, and using the primers
immobilized on the substrate, the primer for
10 elongating the A-strand, and the primer for
elongating the B-strand;

(5) forming a hybridized product of a nucleic
acid corresponding to the A-strand which has been
elongated and amplified as a result of the PCR
15 reactions and bound to the substrate and a nucleic
acid corresponding to the B-strand which has been
elongated and amplified and has not bound to the
substrate; and

(6) detecting the base sequence to be detected
20 by detecting the hybridized product in the respective
primer-immobilized regions in the array.

3. A method of detecting a nucleic acid,
comprising the steps of:

(1) preparing plural single-stranded nucleic
25 acids each having a partial and sequential base
sequence to be detected (A-strand group: A₁-strand to
A_n-strand: $n \geq 2$) and a group of single-stranded

nucleic acids each having a base sequence complementary to a base sequence of each strand of the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);

5 (2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base
10 sequences to be detected are distributed in the primer-immobilized regions;

 (3) preparing nucleic acids each having a sequence complementary to a partial and sequential base sequence within the region between a 3'-end of
15 each strand of the A-strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strands (PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);

 (4) performing PCR reactions using each strand
20 of the A-strand group and each corresponding strand of B-strand group as templates, and using the primers immobilized on the substrate, and the plural primers for elongating the B-strands of the PB-strand group;

 (5) forming a hybridized product of a nucleic
25 acid corresponding to the A-strand group which has been elongated and amplified as a result of the PCR reactions and bound to the substrate and a nucleic

acid corresponding to the B-strand group which has been elongated and amplified and has not bound to the substrate; and

(6) detecting the base sequence to be detected
5 by detecting the hybridized product in the respective primer-immobilized regions in the array.

4. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic
10 acids each having a partial and sequential base sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded nucleic acids each having a base sequence complementary to a base sequence of each strand of
15 the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers
20 independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing nucleic acids each having a
25 partial and sequential base sequence within the region between a 5'-end of each strand of the A-strand group and the base sequence to be detected

which is located nearest the 5'-end as primers for
elongating the A-strands (PA-strand group: PA1-strand
to PAn-strand: $n \geq 2$) and preparing nucleic acids
having a sequence complementary to a partial and
5 sequential base sequence within the region between a
3'-end of each strand of the A-strand group and the
base sequence to be detected which is located nearest
the 3'-end as primers for elongating the B-strands
(PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);
10 (4) performing PCR reactions using each strand
of the A-strand group and each corresponding strand
of the B-strand group as templates, and using the
primers immobilized on the substrate, the primers for
elongating the A-strands of the PA-strand group, and
15 the primer for elongating the B-strand of the PB-
strand group;
(5) forming a hybridized product of a nucleic
acid corresponding to the A-strand group which has
been elongated and amplified as a result of the PCR
20 reactions and bound to the substrate and a nucleic
acid corresponding to the B-strand group which has
been elongated and amplified and has not bound to the
substrate; and
(6) detecting the base sequence to be detected
25 by detecting the hybridized product in the respective
primer-immobilized regions in the array.

5. A method of detecting a nucleic acid

according to any one of claims 1 to 4, further comprising a step of washing and removing a reaction solution on the substrate after the PCR reactions.

6. A method of detecting a nucleic acid
5 according to any one of claims 1 to 4, wherein the primer for elongating the B-strand is labeled, and the hybridized product is detected using the label.

7. A method of detecting a nucleic acid
according to claim 5, wherein the label is a
10 fluorescent dye.

8. A method of detecting a nucleic acid
according to claim 7, further comprising a step of
observing the fluorescent dye using a confocal
fluorescent microscope for detecting the hybridized
15 product.

9. A method of detecting a nucleic acid
according to any one of claims 1 to 4, wherein the
hybridized product is detected using a fluorescent
dye as an intercalator or a groove binder which
20 interacts with a double-stranded nucleic acid.

10. A method of detecting a nucleic acid
according to claim 9, further comprising a step of
observing the fluorescent dye using a confocal
fluorescent microscope for detecting the hybridized
25 product.

11. A method of detecting a nucleic acid,
comprising the steps of:

(1) preparing a single-stranded nucleic acid having plural partial and sequential base sequences to be detected (A-strand) and a single-stranded nucleic acid having a base sequence complementary to
5 a base sequence of the A-strand (B-strand);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and
10 preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing a nucleic acid having a sequence complementary to a partial and sequential base
15 sequence within the region between a 3'-end of the A-strand and the base sequence to be detected which is located nearest the 3'-end as a primer for elongating the B-strand;

(4) performing PCR reactions using the A-strand and the B-strand as templates, and using the primers
20 immobilized on the substrate, and the primer for elongating the B-strand, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to
25 the A-strand which has been elongated and amplified from a primer binding to the substrate via the label

incorporated in the nucleic acid.

12. A method of detecting a nucleic acid,
comprising the steps of:

- (1) preparing a single-stranded nucleic acid
5 having plural partial and sequential base sequences
to be detected (A-strand) and a single-stranded
nucleic acid having a base sequence complementary to
a base sequence of the A-strand (B-strand);
- (2) preparing nucleic acids as primers each
10 having one of the plural base sequences to be
detected, immobilizing the respective primers
independently in separate regions on a substrate, and
preparing a primer array in which the respective base
sequences to be detected are distributed in the
15 primer-immobilized regions;
- (3) preparing a nucleic acid having a partial
and sequential base sequence within the region
between a 5'-end of the A-strand and the base
sequence to be detected which is located nearest the
20 5'-end as a primer for elongating the A-strand and
preparing a nucleic acid having a base sequence
complementary to a partial and sequential base
sequence within the region between a 3'-end of the B-
strand and the base sequence to be detected which is
25 located nearest the 3'-end as a primer for elongating
the B-strand;
- (4) performing PCR reactions using the A-strand

and the B-strand as templates, and using the primers immobilized on the substrate, the primer for elongating the A-strand, and the primer for elongating the B-strand, and nucleotide monomers with
5 a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label
10 incorporated in the nucleic acid.

13. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic acids each having a partial and sequential base
15 sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded nucleic acids each having a base sequence complementary to a base sequence of each strand of the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);
20

(2) preparing nucleic acids as primers each having one of the plural base sequences to be detected, immobilizing the respective primers independently in separate regions on a substrate, and
25 preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

(3) preparing nucleic acids each having a sequence complementary to a partial and sequential base sequence within a region between a 3'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strands (PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);

(4) performing PCR reactions using each strand of the A-strand group and each corresponding strand of the B-strand group as templates, and using the primers immobilized on the substrate and the plural primers for elongating the B-strands of the PB-strand group, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

(5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label incorporated in the nucleic acid.

14. A method of detecting a nucleic acid, comprising the steps of:

(1) preparing plural single-stranded nucleic acids each having a partial and sequential base sequence to be detected (A-strand group: A1-strand to An-strand: $n \geq 2$) and a group of single-stranded nucleic acids each having a base sequence complementary to a base sequence of each strand of

the A-strand group (B-strand group: B1-strand to Bn-strand: $n \geq 2$);

(2) preparing nucleic acids as primers each having one of the plural base sequences to be
5 detected, immobilizing the respective primers independently in separate regions on a substrate, and preparing a primer array in which the respective base sequences to be detected are distributed in the primer-immobilized regions;

10 (3) preparing nucleic acids each having a partial and sequential base sequence within the region between a 5'-end of each strand of the A-strand group and the base sequence to be detected which is located nearest the 5'-end as primers for
15 elongating the A-strands (PA-strand group: PA1-strand to PAn-strand: $n \geq 2$) and preparing nucleic acids each having a base sequence complementary to a partial and sequential base sequence within the region between a 3'-end of each strand of the A-
20 strand group and the base sequence to be detected which is located nearest the 3'-end as primers for elongating the B-strand (PB-strand group: PB1-strand to PBn-strand: $n \geq 2$);

(4) performing PCR reactions using each strand
25 of the A-strand group and each corresponding strand of the B-strand group as templates, and using the primers immobilized on the substrate and respective

primers of the PA-strand group and PB-strand group, and nucleotide monomers with a part or all of at least one group of the nucleotide monomer being labeled; and

- 5 (5) detecting a nucleic acid corresponding to the A-strand which has been elongated and amplified from a primer binding to the substrate via the label incorporated in the nucleic acid.

10 15. A method of detecting a nucleic acid according to any one of claims 11 to 14, further comprising a step of washing and removing a reaction solution on the substrate after the PCR reactions.

15 16. A method of quantitative determination of a nucleic acid based on signals detected according to any one of claims 1 to 4 and 11 to 14.

 17. A method of detecting a nucleic acid according to any one of claims 11 to 14, wherein the label is a fluorescent dye.

20 18. A method of detecting a nucleic acid according to claim 17, further comprising a step of observing the fluorescent dye using a confocal fluorescent microscope for detecting the hybridized product.

25 19. A method of detecting a nucleic acid according to any one of claims 1 to 4 and 11 to 14, wherein at least the PCR reactions and nucleic acid detections are performed in a form in which the

primer arrays are present in the same container.

20. A method of detecting a nucleic acid according to claim 19, wherein the respective PCR reactions and nucleic acid detections are performed
5 while observing intermittently using the same means.

21. An apparatus for detecting a nucleic acid, which enables the method of detecting a nucleic acid according to claim 19, comprising:

a PCR reaction container; and
10 detection means.

22. An apparatus for detecting a nucleic acid according to claim 21,

wherein said PCR container comprises a substrate having a surface with immobilized polymers,
15 a reaction chamber and a temperature controlling unit,

wherein said substrate is transparent against wavelength used for detection

wherein said reaction chamber is facing to said surface,

20 wherein said temperature controlling unit is placed at a position not preventing operation of said detection means, and

wherein said detection means is placed on the side opposite to said surface in relation to said
25 substrate.

23. A kit for detecting a nucleic acid, comprising a primer array; a PCR reaction reagent;

and a nucleic acid detecting reagent, for performing the method according to any one of claims 1 to 4 and 11 to 14.

24. A kit for detecting a nucleic acid
5 according to claim 23, wherein the nucleic acid detecting reagent is a fluorescent dye serving as an intercalator or groove binder which acts on a double-stranded nucleic acid.